

Paragraph spanning page 1, line 33 to page 2, line 6

FATP is a 63-KDa membrane protein isolated from mouse fat cells using the expression cloning strategy. The incorporation of long chain fatty acids was selectively enhanced in animal cells in which the protein was expressed continuously (Shaffer J. E. and Lodish H. F. Cell 79: 427-436, 1994). Subsequent studies revealed the existence of several homologues of this protein, and thus, mouse FATP-1, -2, -3, -4 and -5 were cloned. Out of them, FATP-1, -2, and -5 are full-length, and were confirmed to be active. However, FATP-3 and -4 are not full-length, and thus, the activity has not been confirmed (Hirsch. D. Proc. Natl. Acad. Sci. 95: 8625-8629, 1998).

Paragraph spanning page 3 lines 5-10

Further, FATP family members have AMP-binding motif and conserved C-terminal region in the primary structure, and these regions are also conserved in acyl-CoA synthetase. Accordingly, FATP family members are also predicted to have acyl-CoA synthetase activity. Up to now, mouse FATP-1 has been reported to have this activity (Natalie Ribarik Coe, J.B.C., 274, 36300-36304, 1999).

Paragraph spanning page 3, line 36 to page 4, line 23

Thus, to achieve the above-mentioned objective, the present inventors conducted strenuous studies aiming at the cloning of novel human genes, as follows. First, clones having a high fullness ratio were isolated from a human cDNA library prepared by the oligo-capping method (Maruyama K. and Sugano S. Gene 138: 171-174, 1994; Suzuki Y. et al. Gene 200: 149-156, 1997). The nucleotide sequences of the obtained cDNA clones having a high fullness ratio were then determined from both the 5' and 3' ends. Then, human full-length cDNAs, which were deduced to be full length cDNA clones, were selected by ATGpr (Salamov A. A. et al. Bioinformatics 14: 384-390, 1998; see the ATGpr website of the Helix Research Institute, Japan (<http://www.hri.co.jp/atgpr/>)), and such. Based on the nucleotide sequences of human full-length cDNA clones thus obtained, clones that were predicted by

PSORT (Nakai K and Kanehisa M. Genomics 14: 897-911, 1992) to have a signal sequence were specifically selected. cDNA clones predicted to encode membrane proteins were thus obtained. The full-length cDNA sequences of the clones were analyzed, and the amino acid sequences were deduced from the nucleotide sequences. Then, GenBank (see the GenBank website of the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/Web/Genbank/index.html>)) and SwissProt (see the SwissProt website of European Bioinformatics Institute) (http://www.ebi.ac.uk/ebi_docs/swissprot_db/swisshome.html)) were searched for homology to the amino acid sequences deduced by BLAST (Altschul S. F. et al. J. Mol. Biol. 215: 403-410, 1990; Gish W. and States D. J. Nature Genet. 3: 266-272, 1993; see the BLAST website of NCBI (<http://www.ncbi.nlm.nih.gov/BLAST/>)).

Paragraph spanning page 6, line 25 to page 7, line 1

The homologies of amino acid sequences and nucleotide sequences of the present invention can be determined using BLAST algorithm by Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90:5873-5877, 1993). Based on this algorithm, programs such as BLASTN and BLASTX have been developed (Altschul et al. J. Mol. Biol. 215:403-410, 1990). When nucleotide sequences are analyzed by BLASTN based on BLAST, the parameters may be, for example, score = 100, and word length = 12. Further, when amino acid sequences are analyzed by BLASTX based on BLAST, the parameters may be, for example, score = 50 ,and word length = 3. When BLAST and Gapped BLAST programs are used, default parameters can be used in the respective programs. Specific techniques used in these analytical methods are well known (see the website of NCBI (<http://www.ncbi.nlm.nih.gov.>)).

The two paragraphs spanning page 16, lines 27-35

Figure 2 shows the incorporation of oleic acid into cells transiently expressing PSEC67. The vertical axis indicates the rate of oleic acid incorporation when compared with that into the mock-transfected cells.

Figure 3 shows the incorporation of oleic acid into cells transiently expressing PSEC67. The numerals in the horizontal axis indicate clone numbers. The vertical axis indicates the degree of fatty acid incorporation (fold) relative to that of the mock-transfected clone.

Paragraph spanning page 17, lines 14-19

Figure 10 shows the alignment of the amino acid sequences of PSEC67 of the present invention (top) and human acyl-CoA synthetase (bottom). The sequence underlined, YIFTSGTTGLPK, indicates an AMP-binding motif.

Paragraph spanning page 19 lines 4-11

ATGpr is a program that predicts a translation initiation codon based on characteristics of sequences in the vicinity of ATG codon, which was developed by A. A. Salamov, T. Nishikawa, and M. B. Swindells at the Helix Research Institute (A. A. Salamov, T. Nishikawa, M. B. Swindells, Bioinformatics, 14: 384-390 (1998); see the ATGper website of the HelixResearch Institute, Japan (<http://www.hri.co.jp/atgpr/>)) . The result was shown as an expectation value (0.05-0.94) for when ATG is the true start codon. The ATGpr1 value of PSEC67 was 0.26.

Paragraph spanning page 25, lines 1-35

[Example 5] Assay for the activity of incorporating oleic acid

In order to test the activity of incorporating fatty acids, the incorporation of [¹⁴C]-labeled oleic acid into recombinant cells was examined. As described above, the plasmid was introduced into 293 cells in a 15-cm dish using the calcium-phosphate method or lipofectamine method, and the culture medium was changed with a fresh one on the following day. The culture medium was changed with D-MEM (antibiotics -) containing 10% CS without fatty acid the day before the assay, and the cells were cultured for one day. First, albumin-bound fatty acid was prepared on the day scheduled for the assay. 10 µl of [¹⁴C]-oleic acid was added to 100 µl of Milli-Q water incubated at 40°C, and the mixture was gently stirred. A BSA solution without fatty acids (prepared from fr. V, SIGMA, 20 g/100 ml) was added to the mixture at a final molar ratio of 1:1, and the mixture was stirred gently. Twice as much volume of PBS(-) was added to the mixture, and the resultant solution was incubated at 37°C for 45 minutes. The culture supernatant was sucked off and the cells were washed twice with 5 ml of PBS(-). 5 ml of PBS(-) was added to the dish, and then the cells were scraped off with a cell scraper and transferred to a tube. After the cells were collected by centrifugation, the cell density was adjusted to 5x 10⁵ cells/ml by adding PBS(-) thereto. Then, 200-µl aliquots of the cell suspension were transferred into 15-ml polypropylene tubes on ice. After a pre-incubation of the cell suspension at 37°C for five minutes, 50 µl of albumin-bound fatty acid (albumin-bound [¹⁴C]-oleic acid solution) was added to each tube, and the tubes were incubated at 37°C for 3 minutes. After the reaction, 5 ml of a stop solution (= Washing solution; 0.1% BSA without fatty acid, 200 µM phloretin in PBS (-)) that was pre-cooled on ice was added to each tube. After the reaction was stopped, the solution was filtered through a GF/C glass filter saturated with a saturate buffer (0.1% BSA (without fatty acid) in PBS(-)) overnight, and then the cells were washed 3 times with 5 ml of a washing solution. Then, this glass filter was soaked in a liquid scintillation vial containing 10 ml of Clear-sol 1 (nacalai tesque) overnight, and the radioactivity was measured.

Paragraph spanning page 26, lines 10-15

Further, recombinant cell lines continuously expressing PSEC67 were established by selecting PSEC67-overexpressing clones using G-418. The cell lines were tested for the degree of oleic acid incorporation, and the oleic acid incorporation was revealed to be enhanced significantly as compared with the mock-transfected clone (Figure 3).